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Development of Stability Indicating New RP-HPLC Method and Validation for The Estimation of Rucaparib in Pure Form and Marketed Pharmaceutical Dosage Form

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Abstract

Objective: The current investigation was pointed at developing and progressively validating novel, simple, responsive, and stable RP-HPLC method for the measurement of active pharmaceutical ingredient and Marketed Pharmaceutical Dosage form of Rucaparib. **Methods:** A simple, selective, validated, and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Rucaparib. The chromatographic strategy utilized Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm, using isocratic elution with a mobile phase of Phosphate Buffer (0.02M) and Acetonitrile were consisting of 48:52% v/v (pH-2.80). A flow rate of 1.0 ml/min and a detector wavelength of 248 nm utilizing the UV detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines. **Results:** LOD and LOQ for the two active ingredients were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of R2>0.999, meaning the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness was determined as a part of method validation and the results were found to be within the acceptable range. **Conclusion:** The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of the selected drug.

Keywords
Rucaparib, RP-HPLC, Method Development, Validation, Accuracy, Robustness

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INTRODUCTION

Rucaparib is an orally bioavailable tricyclic indole and inhibitor of poly (ADP-ribose) polymerases (PARPs) 1 (PARP1), 2 (PARP2) and 3 (PARP3), with potential chemo/radio sensitizing and antineoplastic activities. Upon administration, Rucaparib[1] selectively binds to PARP1, 2 and 3 and inhibits PARP-mediated DNA repair. This enhances the accumulation of DNA strand breaks, promotes genomic instability, and induces cell cycle arrest and apoptosis. This may enhance the cytotoxicity of DNA-damaging agents and reverse tumor cell resistance to chemotherapy and radiation therapy. PARPs are enzymes activated by single-strand DNA breaks that catalyze the post-translational ADP-ribosylation of nuclear proteins, which induces signaling and the recruitment of other proteins to repair damaged DNA. The PARP-mediated repair pathway plays a key role in DNA repair and is dysregulated in a variety of cancer cell types. Rucaparib[2] is a small molecule inhibitor of poly

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ADP-ribose polymerase that is used in the therapy of selected patients with refractory and advanced ovarian carcinoma. Rucaparib therapy is associated with a moderate rate of transient elevations in serum aminotransferase during therapy but has not been linked to instances of clinically apparent liver injury. Rucaparib[3] is indicated for the maintenance treatment of adult patients with a deleterious BRCA mutation (germline and/or somatic)- associated recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to platinum-based chemotherapy. The IUPAC Name of Rucaparib is 6-fluoro-2-[4-(methyl amino methyl) phenyl]-3, 10-diazatri cyclo [6.4.1.04, 13] trideca-1, 4, 6, 8 (13)-tetraen-9-one. The Chemical Structure of Rucaparib is shown in Figure-1.

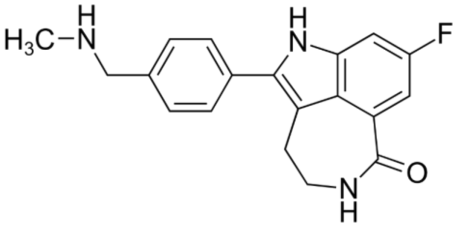

Fig-1: Chemical Structure of Rucaparib

Literature survey[31-33] revealed that Rucaparib was determined in bulk form and marketed pharmaceutical dosage forms by RP-HPLC as well as in biological fluids using liquid chromatography and liquid chromatography mass spectrometric methods. In the present work the authors have developed a simple, rapid, precise, accurate and robust stability indicating[4] liquid chromatographic method for the determination of Rucaparib in pure substances and marketed pharmaceutical dosage forms as per ICH guidelines.

MATERIALS AND METHODS

Table-1: List of Instrument used.

Instruments/Equipments/Apparatus
Waters HPLC with Empower2 Software with Isocratic with UV-Visible Detector.
ELICO SL-159 UV – Vis spectrophotometer
Electronic Balance **(SHIMADZU ATY224)**
Ultra Sonicator **(Wensar wuc-2L)**
Thermal Oven
Symmetry RP C_{18}, 5µm, 250mm x 4.6mm i.d.
P^H Analyzer **(ELICO)**
Vacuum filtration kit **(BOROSIL)**

Table-2: List of Chemicals used.

Name	Specifications		Manufacturer/Supplier
	Purity	Grade	
Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
Methanol	99.9%	HPLC	Loba Chem; Mumbai.
Dipotassium hydrogen orthophosphate	96%	L.R.	Sd fine-Chem ltd; Mumbai
Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
Potassium dihydrogen orthophosphate	99.9%	L.R.	Sd fine-Chem ltd; Mumbai
Sodium hydroxide	99.9%	L.R.	Sd fine-Chem ltd; Mumbai
Hydrochloric acid	96%	A.R.	Sd fine-Chem ltd; Mumbai
3% Hydrogen Peroxide	96%	A.R.	Sd fine-Chem ltd; Mumbai

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum[5] in the range of 200 to 400nm. This has been performed to know the maxima of Rucaparib, so that the same wave number can be utilized in HPLC UV detector for estimating the Rucaparib.

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Rucaparib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.5ml of the above Rucaparib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[11-12].

Preparation of Sample Solution:

Twenty tablets were taken, and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well. A quantity of powder of Rucaparib equivalent to 10mg was transferred to a clean and dry 10 ml volumetric flask and 7 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. One ml (0.5 ml) of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas.

Preparation of 0.02M Potassium Dihydrogen Orthophosphate Solution:

About 2.72172 grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved, and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 2.80 with diluted orthophosphoric acid Solution.

Preparation of Mobile Phase:

480mL (48%) of above Phosphate buffer solution and 520mL of HPLC Grade Acetonitrile (52%) were mixed well and degassed in ultrasonic water bath for 15 minutes. The resulted solution was filtered through 0.45 µm filter under vacuum filtration.

Optimization of Chromatographic Conditions:

The chromatographic conditions[6] were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

RESULTS AND DISCUSSION

Method Development and its Validation for Rucaparib by RP-HPLC

Method Development:

Selection of Wavelength:

While scanning the Rucaparib solution we observed the maxima at 248 nm. The UV spectrum has been recorded on ELICO SL-159 make UV- Vis spectrophotometer model UV-2450. The scanned UV spectrum is attached in the following page,

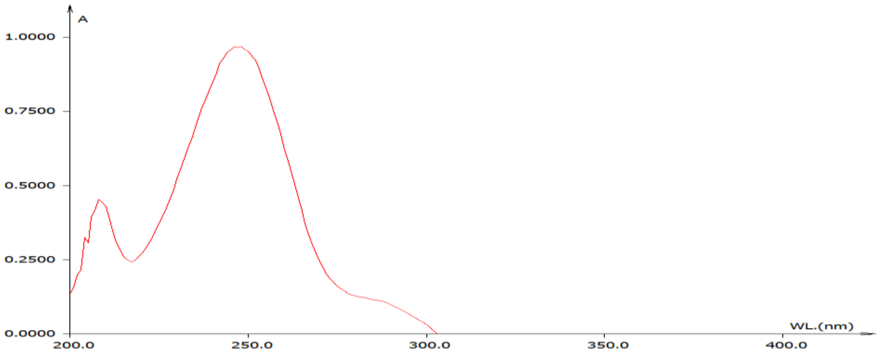

Fig-2: UV Spectrum for Rucaparib

Summary of Optimized Chromatographic Conditions:

The Optimum conditions[7] obtained from experiments can be summarized as below:

Table-3: Summary of Optimised Chromatographic Conditions

Mobile phase	Phosphate Buffer (0.02M): Acetonitrile = 48:52 (pH-2.80)
Column	Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	248 nm
Flow rate	1.0 ml/ min.

Run time	08 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Mode of Elution	Isocratic
Retention time	3.649 minutes

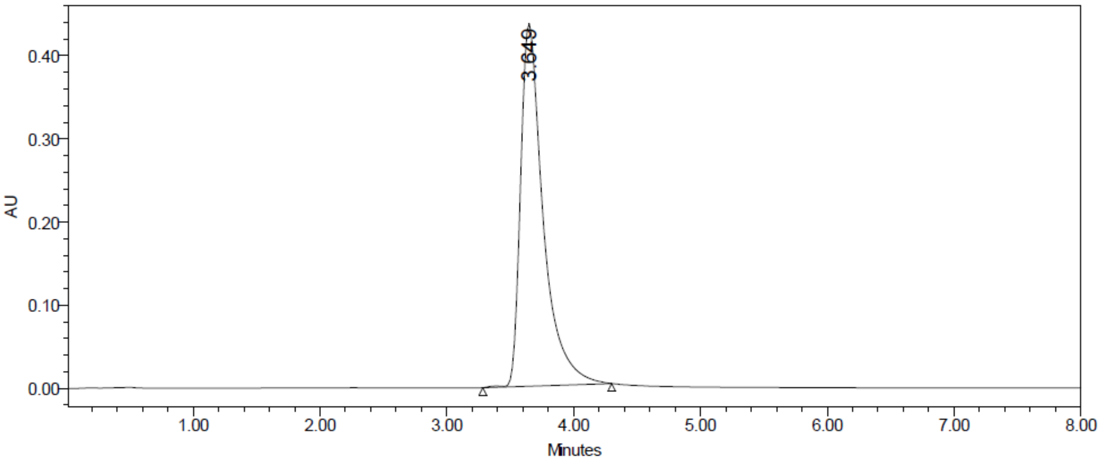

Fig-3: Chromatogram of Rucaparib in Optimized Chromatographic Condition

Validation of Analytical Method

After the chromatographic and the experimental conditions were established, the method was validated by the determination of the following parameters such as specificity, system suitability, linearity, precision, accuracy, robustness, limit of detection (LOD) and limit of quantitation (LOQ) as per ICH Q2 (R1) guidelines[29-30].

1. Accuracy:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Rucaparib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5ml of the above Rucaparib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

For Preparation of 80% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Rucaparib working standard into a 10ml of clean dry volumetric flasks, add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.4ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

For Preparation of 100% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Rucaparib working standard into a 10ml of clean dry volumetric flasks, add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

For Preparation of 120% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Rucaparib working standard into a 10ml of clean dry volumetric flasks, add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.6ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Recovery Study:

To determine the accuracy[8] of the planned technique, recovery studies were distributed by adds completely different amounts (80%, 100%, and 120%) of pure drug of Rucaparib were taken and extra to the pre-analyzed formulation of concentration 50µg/ml. From that proportion recovery values were calculated. The results were shown in table 4.

Table-4: Accuracy Readings

Sample ID	Concentration (μg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	40	40.141	502647	100.352	Mean= 100.3947%
S_2 : 80 %	40	40.191	503214	100.477	S.D. = 0.071319
S_3 : 80 %	40	40.142	502656	100.355	% R.S.D.= 0.071038
S_4 : 100 %	50	50.044	614215	100.088	Mean= 99.98533%
S_5 : 100 %	50	49.887	612451	99.774	S.D. = 0.183045
S_6 : 100 %	50	50.047	614254	100.094	% R.S.D.= 0.183071
S_7 : 120 %	60	60.192	728547	100.32	Mean= 100.311%
S_8 : 120 %	60	59.939	725698	99.898	S.D. = 0.408574
S_9 : 120 %	60	60.429	731211	100.715	% R.S.D.= 0.407308

2. Precision:

2.1. Repeatability

Preparation of Rucaparib Product Solution for Precision:

Accurately weigh and transfer 10 mg of Rucaparib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Procedure:

The standard solution was injected six times and measured the area for all six injections in HPLC[9]. The %RSD for the area of six replicate injections was found to be within the specified limits.

The exactitude of every technique was determined one by one from the height areas & retention times obtained by actual determination of six replicates of a set quantity of drug. Rucaparib (API). The % relative variance was calculated for Rucaparib square measure bestowed within the table 5.

Table-5: Repeatability Readings

HPLC Injection Replicates of Rucaparib	Retention Time (Minutes)	Peak Area
Replicate – 1	3.649	5674158
Replicate – 2	3.684	5654715
Replicate – 3	3.687	5665841
Replicate – 4	3.688	5654578
Replicate – 5	3.688	5652284
Replicate – 6	3.687	5641487
Average		**5657177**
Standard Deviation		**11369.72**
% RSD		**0.200979**

2.2. Intermediate Precision/Ruggedness:

2.2.1. Intra-Day & Inter-Day:

The intra & inter day variation[10] of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Rucaparib revealed that the proposed method is precise.

Procedure:

Analyst 1: The standard solution was injected six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2:

The standard solution was injected six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits[13].

Analyst-1/Intra Day/Day-1:

Table-6: Results of Ruggedness for Rucaparib Analyst 1

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rucaparib	3.687	584968	65982	4985	1.42
2	Rucaparib	3.688	582479	66354	4876	1.46
3	Rucaparib	3.688	586236	67425	4896	1.48
4	Rucaparib	3.687	586985	65982	4986	1.47
5	Rucaparib	3.684	582679	65932	5016	1.45
6	Rucaparib	3.649	583989	65874	4987	1.43
Mean			**584556**			
Std. Dev.			**1846.658**			
% RSD			**0.315908**			

Analyst 2/Inter Day/Day-2:

Table-7: Results of Intermediate Precision Analyst 2 for Rucaparib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Rucaparib	3.649	598698	66985	5265	1.49
2	Rucaparib	3.684	596847	67458	5168	1.47
3	Rucaparib	3.687	596354	66985	5436	1.46
4	Rucaparib	3.688	598676	67854	5369	1.45
5	Rucaparib	3.688	596874	68521	5247	1.48
6	Rucaparib	3.687	598989	67898	5375	1.42
Mean			**597739.7**			
Std. Dev.			**1168.098**			
% RSD			**0.195419**			

3. Linearity & Range:

Preparation of Drug Solutions for Linearity:

Accurately weigh and transfer 10 mg of Rucaparib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5ml of the above Rucaparib stock solutions into a 10ml volumetric flask and dilute up to the mark with Mobile Phase[14].

Preparation of Level – I (30ppm of Rucaparib):

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – II (40ppm of Rucaparib):

Take 0.4ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – III (50ppm of Rucaparib):

Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – IV (60ppm of Rucaparib):

Take 0.6ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – V (70ppm of Rucaparib):

Take 0.7ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Procedure:

Inject each level into the chromatographic system[15] and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

The calibration curve[16] showed good linearity in the range of 0-70µg/ml, for Rucaparib (API) with correlation coefficient (r^2) of 0.999 (Fig-4). A typical calibration curve has the regression equation[17] of $y = 11266.x + 50416$ for Rucaparib.

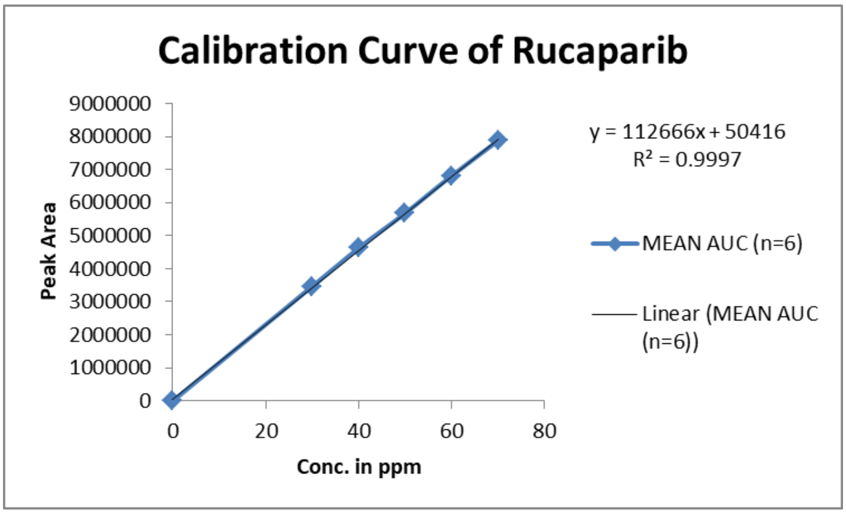

Fig-4: Calibration Curve of Rucaparib (API)

Table-8: Linearity Results

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
30	3465974
40	4626478
50	5682284
60	6815478
70	7878721

Linearity Plot:

The plot of Concentration (x) versus the Average Peak Area (y) data of Rucaparib is a straight line.

Y = mx + c

Slope (m) = 112666

Intercept (c) = 50416

Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 50416. These values meet the validation criteria.

4. Method Robustness:

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Rucaparib. The method is robust only in less flow condition and the method is robust[18] even by change in the Mobile phase ±5%. The standard and samples of Rucaparib were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Rucaparib working standard into a 10ml of clean dry volumetric flasks, add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Effect of Variation of Flow Conditions:

The sample was analyzed at 0.9ml/min and 1.1ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase i.e. Acetonitrile: Phosphate Buffer was taken in the ratio and 40:60, 30:70 instead of 35:65, remaining conditions are same. 10µl of the above sample was injected and chromatograms[19] were recorded.

Table-9: Results for Robustness

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	584624	3.649	1.42	4765
Less Flow rate of 0.9 mL/min	598676	3.687	1.49	4856
More Flow rate of 1.1 mL/min	612543	3.649	1.46	4965
Less organic phase	578642	3.688	1.49	4758
More organic phase	569896	3.684	1.47	4962

5. LOD & LOQ:

LOD: The detection limit[20] of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD = 3.3 × σ / s

Where

σ = Standard deviation[21] of the response

S = Slope of the calibration curve

LOQ: The quantitation limit[22] of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ = 10 × σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Table-10: Results of LOD & LOQ

SE of Intercept	48846.22527
SD of Intercept	109223.4801
LOD	3.199168
LOQ	9.694449

Observation:

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 3.19 & 9.69 µg/ml respectively.

6. System Suitability Parameter:

System quality testing[23-25] is associate degree integral a part of several analytical procedures. The tests square measure supported the idea that the instrumentation, physics, associate degree analytical operations and samples to be analyzed represent an integral system that may be evaluated intrinsically. The following system quality check parameters were established. The information square measured shown in Table-11 & 12.

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Rucaparib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.5ml of the above Rucaparib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

The standard solution was injected six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Table-11: Knowledge of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Asymmetry	T ≤ 2	Rucaparib =0.98
2	Theoretical plate	N > 2000	Rucaparib =4782
3	Tailing Factor	T<2	Rucaparib =1.49

Table-12: Results of System Suitability for Rucaparib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rucaparib	3.644	584635	65847	4857	1.48
2	Rucaparib	3.645	582695	65421	4955	1.42
3	Rucaparib	3.644	587432	65369	4875	1.47
4	Rucaparib	3.662	589687	65748	4796	1.46
5	Rucaparib	3.660	582547	65398	4952	1.49
6	Rucaparib	3.660	589656	652418	4896	1.47

Mean	**586108.7**
Std. Dev.	**3275.654**
% RSD	**0.558882**

7. Specificity:

Specificity[26] can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. Blank solution was prepared by mixing the excipients in the mobile phase without drug. Drug solutions were prepared individually and the sample containing one drug was also prepared. Now these mixtures were filtered by passing through 0.45 µ membrane filter before the analysis. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the proposed method was specific.

The chromatograms representing the peaks of blank, Rucaparib and the sample containing the one drug was shown in following figures respectively.

Observation: In this test method blank, standard solutions were analyzed individually to examine the interference. The above chromatograms show that the active ingredient was well separated from blank and their excipients and there was no interference of blank with the principal peak. Hence the method is specific.

8. Estimation of Rucaparib in Pharmaceutical Dosage Form

Twenty pharmaceutical dosage forms were taken and the I.P. method was followed to work out the typical weight. On top of weighing tablets were finally pulverized and triturated well. A amount of powder cherish twenty five mg of medicine were transferred to twenty five cc meter flask, build and resolution was sonicated for quarter-hour, there once volume was created up to twenty five cc with same solvent. Then ten cc of the on top of resolution was diluted to a hundred cc with mobile part. The answer was filtered through a membrane filter (0.45 µm) and sonicated to remove. The answer ready was injected in 5 replicates into the HPLC system and therefore the observations were recorded.

A duplicate injection of the quality resolution was conjointly injected into the HPLC system and therefore the peak areas were recorded. The info square measure shown in Table-13.

ASSAY:

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak space of drug obtained with check preparation
A = Peak space of drug obtained with normal preparation
WS= Weight of operating normal taken in mg
WT= Weight of sample taken in mg
DS= Dilution of normal resolution
DT= Dilution of sample resolution
P = proportion purity of operating normal

Table-13: Recovery Data for estimation Rucaparib

Brand Name of Rucaparib	Labelled Amount of Drug (mg)	Mean (± SD) Amount (mg) found by the Proposed Method (n=6)	Assay % (± SD)
Nuparp 200 Tab (200mg) (Zydus Oncosciences)	200mg	199.589 (± 0.258)	99.698 (± 0.639)

RESULT & DISCUSSION:

The amount of drugs in Rucaparib Tablet was found to be 199.589 (± 0.258) mg/tab for Rucaparib & % assay[27] was 99.698 (± 0.639).

Forced Degradation Studies

Following convention was entirely clung to for constrained corruption of Rucaparib Active Pharmaceutical Ingredient (API). The API (Rucaparib) was subjected to pressure conditions in different approaches to watch the rate and degree of corruption that is probably going to happen over the span of capacity as well as after organization to body. This is one kind of quickened dependability contemplates that encourages us deciding the destiny of the medication that is probably going to occur after prolonged stretch of time stockpiling, inside a brief timeframe as contrast with the constant or long-haul steadiness testing. The different debasement pathways[28] contemplated are Acid/corrosive hydrolysis, Alkali/fundamental hydrolysis, Thermal/warm Degradation, photolytic corruption/ Degradation, and oxidative Degradation/corruption.

Results of Degradation Studies:

The results of the stress studies indicated the Specificity of the method that has been developed. Rucaparib was stable in photolytic and peroxide stress conditions. The results of forced degradation studies are given in the following table-14.

Table-14: Results of Forced Degradation Studies of Rucaparib API

Stress Condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	98.76	1.24	100.0
Basic Hydrolysis (0.I M NaOH)	24Hrs.	98.63	1.37	100.0
Thermal Degradation (50 ^{0}C)	24Hrs.	93.98	6.02	100.0
UV (248nm)	24Hrs.	98.84	1.16	100.0
3 % Hydrogen Peroxide	24Hrs.	94.61	5.39	100.0

SUMMARY AND CONCLUSION

The analytical method was developed by studying different parameters. First, maximum absorbance was found to be at 248nm, and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm particle size because it was giving good peak. Ambient temperatures were found to be suitable for the nature of the drug solution. The flow rate was fixed at 1.0ml/min because of the good peak area and satisfactory retention time. Mobile phase is Phosphate Buffer (0.02M) and Acetonitrile were taken in the ratio of 48:52 % v/v (pH-2.80) was fixed due to good symmetrical peak. So, this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. The run time was selected to be 8.0 min because the analysis gave peak around 3.649min and to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision were found to be accurate and well within range. The analytical method was found linearity over the range of 30-70ppm of the Rucaparib target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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